Research article Available online www.ijsrr.org ISSN: 2279–0543

International Journal of Scientific Research and Reviews

Green synthesis of Silver Nanoparticles using *Rauvolfia serpentina* Roots and Screening for their Antimicrobial activity

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ABSTRACT

In the present study silver nanoparticles (AgNPs) were green synthesized using aqueous root extract of *Rauvolfia serpentina* as reducing agent.The amalgamated solution of plant extract and silver nitrate turned in to reddish brown in colourafter 48hrs incubation indicating the formation of AgNPs. Later the synthesized AgNPs were studied in UV-Visible spectroscopic analysis. A strong spectrum was formed with absorption maximum at 436.18nm confirming the formation of AgNPs. Later the green synthesized AgNPs were purified and characterized usingX-ray diffractometer, Flourescencetransform infrared spectroscopy and Scanning electron microscopy.Further the green synthesized AgNPs were screened for their antimicrobial activity against selected pathogenic bacteria and fungi using Kirby-Bauer method. The AgNPs have shown potential antibacterial activity against multidrug resistant human pathogenic bacteria and fungi with maximum zone of inhibition on *Salmonella paratyphi* among bacteriaand *Candida krusei* amongfungi.

KEY WORDS: Green synthesis, *Rauvolfia serpentina*, AgNPs, XRD, Antimicrobial activity

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INTRODUCTION

Metallic nanoparticles are formulated generally by physical, chemical and biological methods. However biological amalgamation of metallic nanoparticles (MNPs) achieved eminence over physical and chemical methods because of its eco friendly and cost effective nature. In biosynthesis MNPs are harvested by using extracts of bacteria, fungi, algae and plants as reducing agents[1]. Green synthesis is a sector of biosynthesis in whichMNPs are formulated using the extracts of plants. Stable nanoparticles were formed in green synthesis because abundant phytochemicals or secondary metabolitespresent in plant extract acts as reducing and capping agents of MNPs. Moreover the reduction of metal ions in to nanoparticles in presence of plant extracts is much faster when compared to other biological extracts[2, 3].

According to research literature till date different types of metals like Platinum (Pt), Gold (Au), Silver (Ag) and Zinc (Zn) were practiced for the formulation of nanoparticles by green route method[4].Among different types of metals, silver is mostly used in green amalgamation of metallic nanoparticles because of its antimicrobial activity. Silver in its pure form is tremendously toxic to microbes and when converted in to nanolevel its activity increases several times. In addition silver is non lethal to humans and when tested in the treatment of various diseases it lefts no allergic reactions[5]. Silver nanoparticles (AgNPs) are widely adopted in biosensing, drug delivery, catalysis, pharmaceuticals as well as in cosmetics because of their unique physicochemical and biologicalproperties[6].

*Rauvolfia serpentina*is an indigenous plant of Indian subcontinent and East Asia, commonly called Indian snake root or Sarpagandhi and belongs to the family Apocyanaceae[7]. The plant encompasses various secondary metabolites like alkaloids, phenols, saponins, flavanoids, glycosides, terpenes and tannins in its parts. The extracts of Sarpagandhi are used in the medication of various disorders like high blood pressure, traumas and epilepsy. The root extract of the plants are usually used as antidote to snake bite. The extracts of this plant have been displayed antibacterial, antifungal, anti inflammatory andantiproliferative activities[8, 9].In the present study silver nanoparticles were synthesized using aqueous root extract of *R.serpentina*a s reducing agent and characterized using different advanced techniques. Further the green synthesized AgNPs of *R.serpentina* were used to screen their antibacterial and antifungal activities.

MATERIALS AND METHODS

Collection of the plant material

The roots of the plant *Rauvolfiaserpentina*were gathered in the forest of Tirumala hills, Tirupathi, India. The plant was taxonomically identified and authenticated by Prof. M.Vijayalakshmi, Dean and Professor, Dept of Botany, AcharyaNagarjuna University, Guntur, Andhra Pradesh, India.

Preparation of the aqueous root extract

The collected plant material was cleanedwith distilled water thrice, lacerated in to pieces and squeezed in to powder with a suitable pulveriser. 3 grams of finely squeezed and dried powder of roots was added to 100ml of molecular grade water, boiled at 100^0C for 10 minutes and filtered with Whatman No 1 filter paper to get the extract.

Biosynthesis of AgNPs from aqueous root extract of R. serpentina

1ml, 5ml, 10ml, 15ml and 20ml of plant extract was combined to 199ml, 195ml, 190ml, 185ml and 180ml of 1mM silver nitrate ($AgNO_3$) solution, stirred using a magnetic stirrer for 10 minutes and keptfor incubation at room temperature. The transition in the colour of the amalgamated solution was examined after 48hrs. The effect of$AgNO_3$ concentration on AgNPs synthesis was also investigated by adding 15ml of plant extract to 0.1mM, 0.5mM, 1mM, 1.5mM and 2mM concentrations of $AgNO_3$ in separate reactions and transition in the colour of reactions was examined after 48hrs.

Characterization of green synthesized AgNPs

The formation and stability of *R.serpentina* root mediated silver nanoparticles was approved by UV-Visible spectroscopic studies after 48hrs using $AgNO_3$ solution as blank.To know the effect of time on AgNPs formation the amalgamated solution containing 15ml of plant extract and 185ml of $AgNO_3$was analyzed using UV-Visible spectrophotometer for every 1hr time intervals. Spectral analysis of the AgNPs was carried using UV-VIS Double beam spectrophotometer (Thermo Fischer) and the values were documented within the range of 200 to 800 nm.The AgNPs of *R. serpentina* were purified from their solution by repeated centrifugation at 10,000 rpm for 15 min and pellet was transferred into a china dish and subjected for the shade evaporation. The dried particles were washed with distilled water and subjectedfor shade drying repeatedly thrice. The purified and dried sample was collected and used for further characterisation. The sample was investigated in Philips X'pert pro XRD with an operation voltage of 40KV and current of 30mA with CuKα radiation

(1.540 °A) between 2θ° angles (30°-80°) for analysing peak data and the crystal structure. FTIR analysis of the AgNPs was carried out through potassium bromide (KBr) pellet (FTIR grade) method in 1:100 ratio and spectrum was documented using Jasco FT/ IR-6300 FTIR equipped with JASCO IRT-7000 Intron Infrared microscope using transmittance mode operating at a resolution of 4 cm^{-1} (JASCO, Tokyo, Japan). Scanning electron micrographs of the purified and dried silver nanoparticles were taken using Zeiss SEM machine. Thin films of the sample were formulated on a glass slide by just dropping a very small amount of the sample on the grid. Then the slide was subjected to dry by putting it under a mercury lamp and SEM images were taken after 10 minutes drying.

Antimicrobial activity of R. serpentinaAgNPs

The test microorganisms Gram positive bacteria *Staphylococcus aureus* (MTCC 3160),*Bacillus subtilis* (MTCC 8364), *Bacillus megaterium* (MTCC 7192); Gram negative bacteria *Escherichia coli* (MTCC 1683), *Pseudomonas aeruginosa*(MTCC 7925), *Salmonella paratyphi* (MTCC 3220); pathogenic fungi *Candida krusei*(MTCC 9215) and *Candida tropicalis* (MTCC 6192) were procured from Microbial type culture collection (MTCC), Chandigarh, India. Kirby-Bauer method was pursued for antimicrobial activity testing as per the recommendations of 2011 Clinical and laboratory Standards Institute guidelines. The bacterial and fungal suspensions of 100µl were spread on the Mueller-Hinton agar plate and Potato Dextrose agar medium respectively. The surface of the agar plates were punched using cork borer. The wells were filled with 100µl of*R.serpentina*AgNPs solution (AgNPs mixed with deionized water) and ampicillin (100µg/ml) as standard in Mueller-Hinton agar plates and Girseoflavin (100µg/ml) as standard in potato dextrose agar plates. The plates were incubated at 37°C for 24hrs for bacteria and 72hrs for fungi. Zone of inhibition was measured to resolve the antimicrobial property of plant mediated AgNPs. Control plates were maintained for contamination monitoring during the test[10].

RESULTS AND DISCUSSION

The inclusion of *R.serpentina*root extract to$AgNO_3$ solution led to the transition in the colour from light yellow to yellowish brown and finally to reddish brown after 48hrs incubation (Fig. 1d) indicating the completion of AgNPs synthesis reaction due to excitation of surface plasmon vibrations[11, 12].Surface plasmon resonance (SPR) is an incredible optical aspect displayed by nano silver particles due to vibration of the conducting metal surface electrons in resonance with the non-particulate radiation. This property is governed by physical properties such as particle type, size, shape and also the provincial chemical effect.

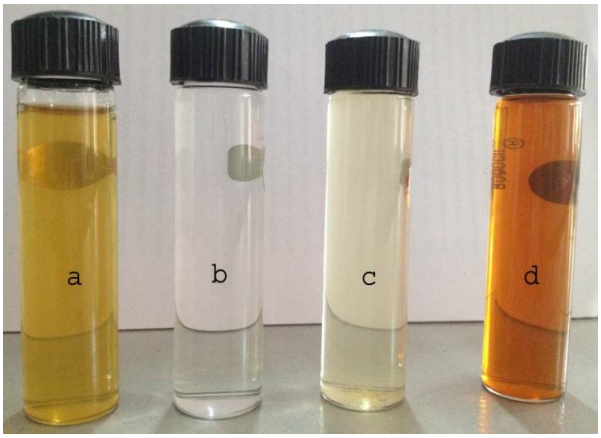

Fig. 1 Green synthesis of silver nanoparticles (a) *R. serpentina* extract
(b) $AgNO_3$ solution (c) A+B at the start of incubation (d) *R. serpentina*AgNPs

Characterization of plant mediated AgNPs

UV-Visible spectroscopy analysis

SPR band for the plant mediated AgNPswas attained with absorption maximum at 436.18nm[13, 14]in UV-Visible analysis after 48hrs incubation (Fig. 2c).When the amalgamated solution kept for incubation to know the effect of plant extract concentration investigated in UV-Visible spectrometer after 48 hrsAgNPs formation was discovered in reactions with 5ml, 10ml, 15ml and 20ml of plant extract only (Fig.2a). Laterthe reactions kept for incubation to know the effect of $AgNO_3$ concentration were examined in UV-Visible spectrometer after 48hrs incubation,AgNPs formation was discovered only to 1mMand 1.5mM concentrations of $AgNO_3$ as shown in the Fig.2b. When the amalgamated solution of 15ml plant extract and 185ml of $AgNO_3$ solution studied in UV-Visible spectrophotometer for every 1hr time intervals the AgNPs formation was witnessed exactly after 12hrs incubation(Fig. 2c).

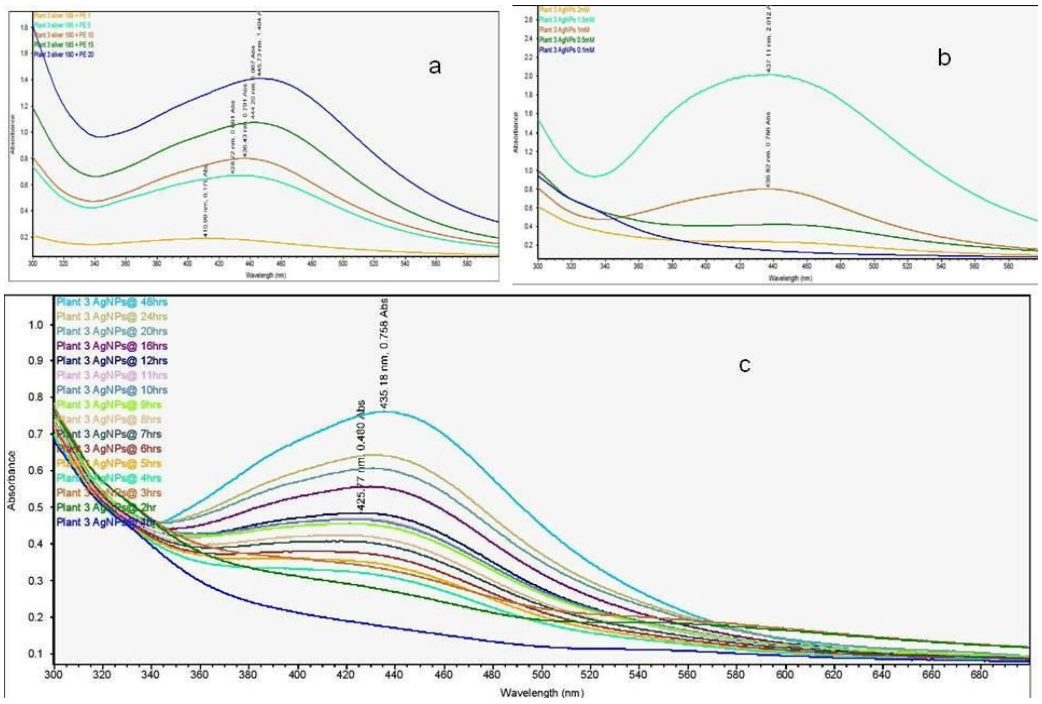

Fig. 2 UV-Visible analyses of (a) Effect of plant extract concentration

(b) Effect of $AgNO_3$ concentration (c) Influence of time on the formation of *R. serpentina*AgNPs

Fourier transform infrared (FTIR) analysis

FT-IR spectroscopy measurements were carried out to diagnose the functional groups of phytochemicals present in the plant extract that are specifically bound to the silver surface as molecular environmental capping agent on the nanoparticles. The FTIR spectrum (Fig. 3a) of the *R.serpentina*AgNPs has shown major peaks at 3354.56cm^{-1}, 2926.01cm^{-1}, 1639.49cm^{-1}, 1384.89cm^{-1}, 1361.74cm^{-1}, 1145.72cm^{-1}, 1035.77cm^{-1}, 833.25cm^{-1} and 746.45cm^{-1}. The strong and broad peak in the spectrum at 3354.56cm^{-1} is due to the OH stretch of alcohols and phenols. The peak at 2926.01cm^{-1} which is strong and medium shows the C-H stretch of alkanes[15]. Astrong and medium peakat 1639.49cm^{-1}was formeddue to the N-H bend of the amides. There were two strong and medium peaks formed at 1384.89cm^{-1} and 1361.74cm^{-1} shows the presence of alkanes and alkyl groups[16]. The strong and medium peaks formed at 1145.72cm^{-1} and 1035.77cm^{-1}are formed due to the C-O stretch of alcohols. Lastly two main peaks observed at 833.25cm^{-1} and 746.45cm^{-1} shows the presence of =C-H bend of alkenes and C-H bend of aromatic compounds respectively[17]. From the FTIR spectrum it can be proclaimed that the secondary metabolites present in the root extract of *R.serpentina* played as reducing and stabilizing agents in the synthesis of silver nanoparticles.

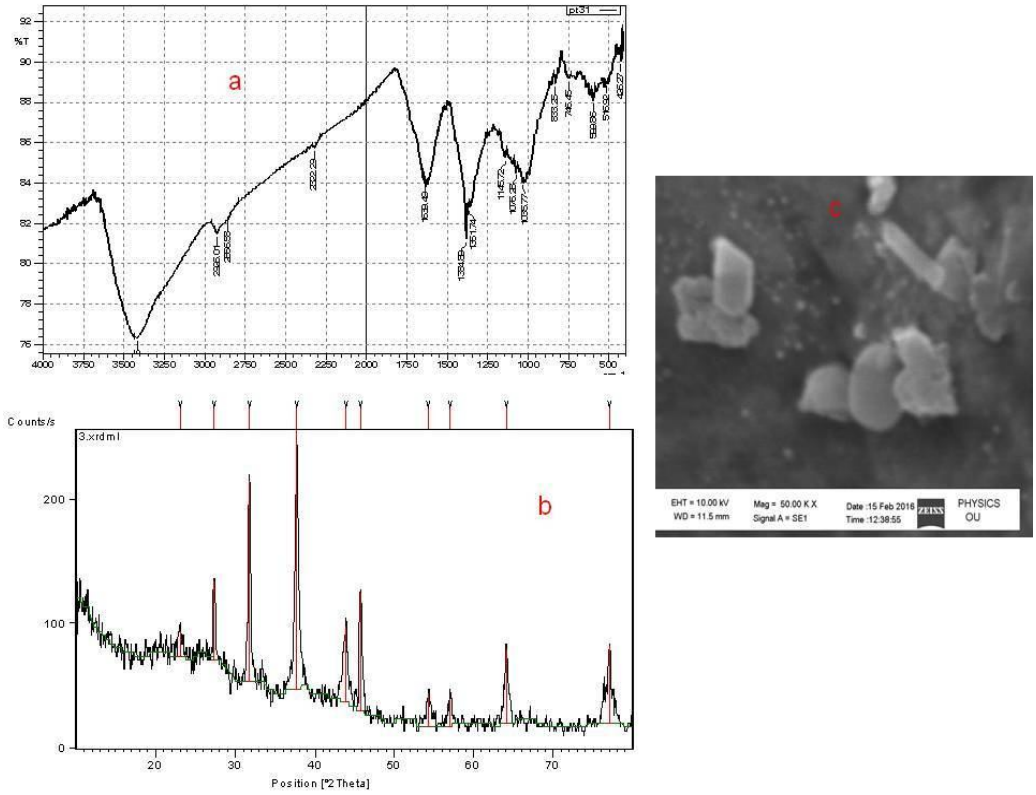

Fig. 3 (a) FTIR spectrum (b) EDX spectrum (c) SEM image of *R. serpentina*AgNPs

X-ray diffractometer analysis

XRD diffractogram of *R. serpentina*AgNPshas shown the Bragg peaks (angle 2θ) at 27.33°, 31.76°, 37.69°, 45.78°, 54.30°, 57.04°, 64.11° and 77.04° (Fig. 3b) which corresponds to the indexed planes of 210, 122, 111, 200, 142, 241, 220 and 311 that demonstrate the formation of face-centered cubic (FCC) AgNPs. Due to crystallization of the bioorganic phases on the surface of the AgNPs, many exceptional peaks were displayed. Similar reports for XRD were shown with the AgNPs incorporated from *Jatrophacurcas*seed extract and *Solanumtuberosum*rootextract[18, 19]. The average particle size of silver nanoparticles formulated in the present study can be calculated using the Debye-Scherrer equation [D = K λ / β cosθ], where D is the mean diameter of the particle, λ is the wavelength of the X-ray source (0.1541 nm) used in XRD, β is the full width at half maximum of the diffraction peak and K is the Scherrer constant with a value 0.9. The approximate size obtained from the XRD data and the Debye-Scherrer equation is 16.25nm for the AgNPs synthesized.

SEM analysis

The SEM image of the green synthesized AgNPs of*R. serpentina* root extract (Fig. 3c) revealed that the silver nanospecks were spherical to non spherical in shape. The nanoparticles were

not aggregated i.e. mono dispersed in nature[20]. The image also proclaimed that the green synthesized AgNPs of *R. serpentina* were in different sizes ranging from 1-100nm.

Antimicrobial activity by well diffusion method

Recent literature proclaimed that the AgNPs display antimicrobial effect by binding to the cell membrane and amend respiration and cell functions. In addition AgNPs also invade in to the bacteria and fungi and interacts with sulphur containing proteins in themicrobial membrane andphosphorus containing compounds like DNA[21]. In the present study the root mediated AgNPs of *R. serpentina* have exhibited strong antimicrobial activity against multi drug resistant gram positive and gram negative bacterial strains as well as selected human pathogenic fungi (Fig. 4). Zone of inhibition of AgNPs against all the pathogens used in the investigation was recorded and depicted in the table. Maximum zone of inhibition was exhibited against *Salmonella paratyphi*and *Candida krusei*among bacteria and fungi respectively. Moreover the green synthesized AgNPs were shown high inhibitory action when compared with the aqueous root extract and $AgNO_3$ alone[22, 23].

CONCLUSION

Green synthesis of metallic nanoparticles emerged as a major research area in the field of nanobiotechnology because of their potential biomedical applications. In the present study silver nanoparticles were green synthesized using dry root powder of *R.serpentina.*When the 15ml plant extract was added to 185ml of 1mM $AgNO_3$ solution and incubated for 48hrs the amalgamated solution turned reddish brown in colour.In UV-Visible spectroscopic analysis a strong spectrum with absorption maximum was formed at 436.18nm confirming the formation of AgNPs. FTIR studies confirmed the presence of secondary metabolites or phytochemicals in the root extract and their reducing and capping activity in the formation of AgNPs. While XRD analysis proclaimed the face centered cubic nature of *R.serpentina*AgNPs. From SEM analysis it can be known that the green synthesized AgNPs were in different shapes i.e. from spherical to irregular and mono dispersed.In the invitro antimicrobial studies the AgNPs were exhibited remarkable activity against all bacteria and fungi used and recorded maximum zone of inhibitionagainst *Salmonella paratyphi*and *Candida krusei*among bacteria and fungi respectively.

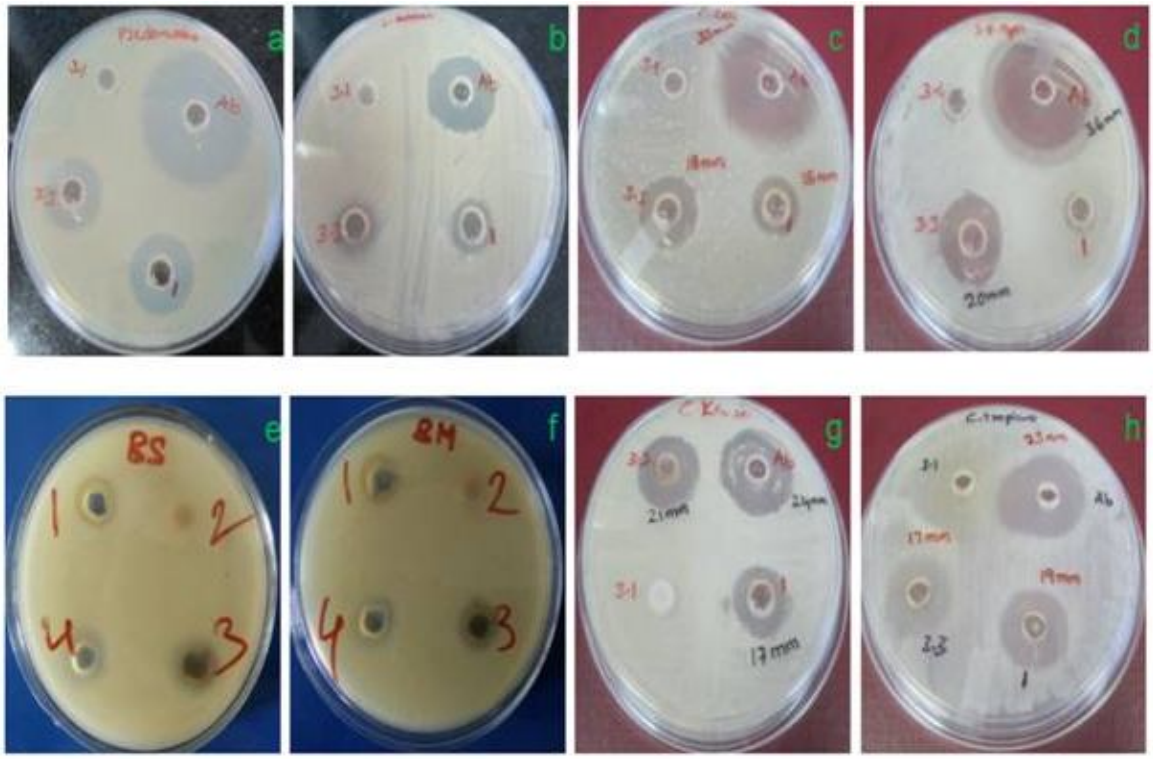

Fig. 4 Antimicrobial activity of *Rauvolfiaserpentina*AgNPs on (a) *Pseudomonas aeruginosa* (b) *Staphylococcus aureus* (c) *Escherichia coli* (d) *Salmonella paratyphi*(e) *Bacillussubtilis* (f) *Bacillusmegaterium*(g) *Candida krusei*(h) *Candida tropicus*

Table: Antimicrobial activity (Zone of inhibition in mm) of *R. serpentina*AgNPs

Name of the microbe	Root Extract	$AgNO_3$	AgNPs	Standard Antibiotic
Pseudomonas aeruginosa	0	18	19	34
Staphylococcus aureus	0	12	12	25
Escherichia coli	0	15	18	33
Salmonella paratyphi	0	0	20	36
Bacillussubtilis	0	0	12	10
Bacillusmegaterium	0	0	12	09
Candida krusei	0	17	21	24
Candida tropicus	0	19	17	23

Values are mean ±S D (n=3)

The above components are subjected to one-way analysis of variance (ANOVA) tested 5% level of significance. All the values are found to be significant ($p<0.05$).

ACKNOWLEDGMENTS

The authors are thankful to UGC for providing RGNF Fellowship to the first author, Department of Biotechnology and Department of Physics, AcharyaNagarjuna University, Guntur for providing research facilities.

Conflict of interest: All the authors declare that there is no conflict of interest

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